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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. — No. 147.

THE COLOR CHANGES IN THE SKIN OF THE SO-CALLED FLORIDA CHAMELEON, ANOLIS CAROLINENSIS Cuv.

By Frank C. Carlton.

With a Plate.

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Contents.

I. Introduction.

Although from the earliest times the color changes of the African chameleon have attracted the attention of naturalists, a wholly satisfactory explanation of these changes has not yet been worked out. This is probably due to the fact that the color changes in this lizard are very likely the most complex of any in the reptiles, and that in consequence their elucidation is proportionally more difficult. As Thilenius ('97) has intimated, it would seem wisest to study first some of the simpler examples of color change, and thereby obtain a clue to the explanation of those in the more complex forms, like the chameleon, rather than to take up the most complex first. In pursuance of this idea I have undertaken the study of the color changes in the skin of the so-called Florida chameleon,

Anolis carolinensis Cuv. This lizard belongs to the family Iguanidae Cope (:00), and thus is not a true chameleon, although, as its popular name indicates, it possesses remarkable properties in the matter of the color changes of its skin.

A large number of living specimens of Anolis were obtained during the winter of 1902–03 from a dealer in Jacksonville, Florida. They lived well in confinement and exhibited characteristic color changes. As Lockwood ('76, p. 12) has already observed, they are as a rule dark brown during the day and pea-green at night. These extremes, with a series of transitional tints running through shades of brown and yellow to green, were the chief colors regularly noticed. Whether these changes, which were observed during the winter in the laboratory, are also characteristic of the animals in their natural haunts, I am unable to say. As far as I have been able to observe, the play of colors so conspicuous in the African chameleon is rarely if ever approached in Anolis. Lockwood ('76, p. 13), however, has described conditions which more nearly resemble those of the African chameleon than any I have seen. In this respect Anolis is much simpler, and consequently much more satisfactory for experimental work, than the true chameleon.

To insure precision in my work, I not only chose a lizard with a relatively simple color change, but I experimented as a rule only on definite portions of its skin, namely, the uniformly tinted areas which cover the sides of the animal's body. These regions showed the full range of color change from dark brown to pea-green, and proved to be convenient areas to deal with. Unless otherwise stated, what is contained in the following account refers to the skin of these regions. I shall begin with a description of the histology of the skin in the brown and in the green states, and I shall afterward take up the physiology of the changes concerned in the production of these two states.

The subject of this research was suggested to me by Dr. G. H. Parker, under whose guidance I have carried out the work.

II. The Histology of the Color Changes.

The skin of Anolis carolinensis, like that of other reptiles, consists of a relatively thin epidermis, in which a horny layer (Fig. 1, *st. crn.*) and a mucous layer (*st. muc.*) can be distinguished, and a derma (*drm.*), composed chiefly of interlacing connective-tissue bundles. Among other structures the derma contains numerous chromatophores, and other less clearly definable pigment masses. The skin is broken up into very

clearly marked scutes, in which all the layers of the skin are considerably thickened, the small tracts between the scutes being the thinnest portions of the skin. The pigment is restricted to the regions under the scutes, and in this respect Anolis resembles more or less closely the chameleon as described by Brücke ('52) and Keller ('95).

So far as the color changes in Anolis are concerned, the active operations seem to be limited to the pigment of the derma, for in the epidermis I have sought in vain for any signs of alteration. In the following description I shall therefore confine my attention to the pigment-bearing organs of the derma.

Keller ('95, p. 141), in his account of the skin of the chameleon, has described five kinds of pigment bodies differing from each other essentially in the kinds of pigment that they contain. All the pigment bodies in the skin of Anolis are easily referred to two, or at most three, of these five kinds. The conspicuous black bodies of Anolis (Fig. 1, *mela'ph.*), well buried in the derma and sending branching processes outward toward the epidermis, correspond to the melanophores described by Keller. The material (Fig. 1, *och'ph.*) that fills in the spaces between the processes of the melanophores is bluish-green by reflected light, and corresponds both in position and character to Keller's layers of ochrophores. It is possible that occasionally a deeper reflecting layer of whitish material corresponding to what Keller calls the leucophore layer may be present, but this is certainly exceptional in Anolis, if in fact it occurs in this lizard at all. The two remaining types of pigment bodies in the chameleon, erythrophores and xanthophores, were not identified in Anolis.

Since the skin of Anolis presents two extreme conditions, dark brown and pea-green, and since the active changes by which these conditions are produced are limited to the two sets of pigment bodies in the derma, I shall restrict the remainder of my account of the skin to these bodies, beginning with the ochrophores.

The ochrophore layer (Fig. 1, *och'ph.*) consists of pigment masses arranged in several irregular rows parallel with the surface rather than in columns perpendicular to it, as in the chameleon.

The physical properties displayed by this pigment in its relation to light are of interest. When a section of skin is viewed under the microscope with transmitted light, this pigment appears yellowish with a slight tinge of green; in reflected light it has a bright greenish-blue tint, suggestive of the color assumed by the animal at night.

Since the ochrophore pigment is readily dissolved in mineral acids and is doubly refractive, as may be demonstrated under the polarizing micro-

scope, it is in all probability an inorganic crystalline deposit. I have not been able to make out its relation to cells. The pigment occurs in blocks, and it is easy to see that nuclei are scattered irregularly between these. The nuclei are similar to those figured by Keller ('95, Taf. 4, Fig. 10) for the ochrophores of the African chameleon, and they may belong to the cells which produce what I have called the ochrophore pigment; but whether this pigment is in the cells or in intercellular spaces I have not been able to determine.

The ochrophore pigment showed no differences in the brown and the green states of the skin, so far as I could observe. The pigment masses did not change in position, the blocks were neither nearer together nor farther apart, and the physical properties remained the same.

The relation of the melanophores to the ochrophore layer is best seen in a section of brown skin parallel to the surface. In such a section (Fig. 2) the ochrophore layer (*och'ph.*) has the appearance of a more or less homogeneous mass, irregular in outline and penetrated in many places by the processes of the melanophores. These processes spread out irregularly over the distal surface of the ochrophore layer.

The melanophores (Fig. 1, *mela'ph.*) occur under the scutes, but not in the spaces between them, and are rather uniformly distributed to the number of about one hundred to a scute. The melanophores are for the most part round or oval in outline. They have a sharply marked contour, and the black pigment that they contain is in the form of small granules. Chlorine, when applied to sections by Mayer's method, destroys the color in these granules. Depigmented sections stained with Delafield's haematoxylin show that each melanophore contains a nucleus, thus demonstrating its cellular nature.

In the dark-brown skin (Fig. 1) each melanophore gives rise on its distal surface to some six or seven processes which extend distally to the region immediately under the epidermis. Near the body of the melanophore the processes show very few branches; but as they approach the epidermis they divide into smaller and smaller branches, which mingle together and form a dense interlacement; in a section perpendicular to the surface of the skin (Fig. 1) these branches seem to form an almost continuous black band. I have seen no evidence that the finer branches anastomose, for in sections of the skin parallel to its surface (Fig. 2) the processes and branches appear as a great number of dots of varying sizes without any connecting network. The processes and their branches are everywhere crowded with black pigment granules like those in the body of the melanophores.

The melanophores in the green skin (Fig. 3, *mela'ph.*) differ from those in the brown skin in that their branches seem to have disappeared, the main processes terminating in rounded ends. Hitherto it has been a question whether the branches are really drawn in like the pseudopodia of an amoeba, or whether the pigment granules simply move down the branch into the process, or into the cell, thus making it difficult to see the transparent branch. The latter condition is now held to be true for the chameleon, as was first maintained by Brücke (**'52**, p. 201). In attempting to ascertain the condition in Anolis I partially depigmented some sections of skin and stained them in Delafield's haematoxylin. In these sections I could see clearly the empty branches extending out from the region where the pigment ceased, to the base of the epidermis, and I was thus convinced that the branches are not withdrawn, but simply emptied of pigment. In the green condition of the skin the cell body of the melanophore, as might be expected, is more densely filled with pigment granules than in the brown state.

It is clear from the foregoing account that the active pigment changes accompanying the color changes in the skin of Anolis occur only in the melanophores. In this respect these changes are like those in Varanus, Uromastix, and Agame as described by Thilenius (**'97**, p. 532). In the dark brown state the pigment of the melanophores fills the processes and branches of these cells to their distal extremities, thus producing an almost continuous dark layer immediately under the epidermis and external to the ochrophore layer. It is this dark layer that gives to the animal its dark color. In the green state the dark pigment of the outer branches of the melanophores has retreated into the deeper processes, or even into the cell body beneath the ochrophore layer, thus exposing this layer to the light. As microscopic preparations show that the material of the ochrophore layer reflects a bright bluish-green light, it is probable that the pea-green color characteristic of this state is due chiefly to reflexion from this layer, though the transparent epidermis may modify more or less the color that would otherwise appear.

The outward migration of the melanophore pigment granules of Anolis in the light and their inward migration in the dark is like that recorded by almost all observers for the African chameleon. It is the reverse of the condition described for Stellio by Filippi (**'66**), for Phrynosoma by Wiedersheim (Hoffmann, **'90**, p. 1353) and for Varanus, Uromastix, and Agame by Thilenius (**'97**). In all these reptiles the melanophore pigment moves *inward* in the *light*, and *outward* in the *dark*.

III. The Physiology of the Color Changes.

1. *Introductory.* — As previously stated, Lockwood ('76) has observed — and I can abundantly confirm his observations — that Anolis carolinensis when in confinement during the winter is usually dark brown by day and pea-green at night. That this change is not simply rhythmic in character, but is dependent upon factors in the environment, is seen from the following experiments. When an Anolis that has turned green at night is placed in a dark-box so that it remains in the dark after day-break, it retains its green color until exposed to daylight, whereupon it rapidly turns brown. If, on the other hand, a brown Anolis is exposed to

TABLE I.

Individual.	Time in Minutes required to change from	
	Dark Brown to Green.	Green to Dark Brown.
No. 1	26	4
" 2	22	3
" 3	36	2
" 4	23	8
" 5	17	2
Average	25	4

illumination from a strong arc light (a gas-light is not sufficient) as evening comes on, it retains its former color and does not become green as long as it remains in a strong light. Thus the color changes are not rhythmic in correspondence with day and night, but depend upon the immediate effects of some factor in the environment, presumably light.

When a brown Anolis is put in the dark it invariably becomes green. This experiment I have repeated with perhaps one hundred animals, each one being tried several times, and I have never found an exception to this rule. The absence of light is therefore a means of inducing the green state.

When a green Anolis is put into the light it almost invariably becomes brown. I have repeated this experiment many times, and I have only

rarely found individuals which retained their green color in the light for any great length of time. All such individuals, moreover, have eventually become dark brown, though in extreme cases the change has not occurred until the animal had been exposed to the light as long as three hours. Excepting these few instances, in which there has been a temporary retardation of the brown state, it may be stated that generally daylight induces the brown condition.

The changes from brown to green and from green to brown are, however, by no means reversed repetitions of each other. The rate of change differs according to its direction; in Table I are given a few characteristic rates. As this table shows, the change to brown is accomplished much more rapidly than that to green. However, a few individuals were found in which the two rates were nearly equal; thus in one instance the change to green was accomplished in 14.5 minutes, that to brown in 13.5 minutes. Such cases as these are, however, rare, the great majority following the general rule indicated in Table I.

The chief facts thus far observed may be summarized as follows: The dark brown condition of the skin is produced by the outward migration of the pigment of the melanophores, a process which takes place in the light and requires on the average about four minutes for completion. The green condition is produced by the inward migration of the melanophore pigment whereby the ochrophore layer is exposed to the light, a process which takes place in the dark and requires on the average about twenty-five minutes for completion.

2. *Brown Condition.* — I shall now endeavor to make clear the factors which are concerned with these two changes, beginning with the change from green to brown. This change is, as I have said before, almost always accomplished quickly when a green animal is exposed to daylight. Under these conditions it is conceivable that the change may be due either to the direct action of the light on the melanophores or to changes induced in these cells through the nervous system, which in its turn is stimulated by light.

My first experiments were directed to finding out whether the illumination of one part of the body had any influence on the color changes in those parts which were not illuminated. To determine this I used a dark-box about six inches long, two inches high, and five inches broad. The box was blackened inside and was provided with a movable lid. At one end of the box a small hole was made sufficiently large to admit the head of a lizard of average size. The hole was surrounded by a collar of loose black cloth, which could be made to fit snugly to the neck of the

animal and thus hold it in place as well as keep light from entering the box. The lizard could be placed either with the head projecting out of the box and in daylight, while the trunk remained in the box in darkness, or the reverse.

To test the possible effects of this apparatus independent of light conditions, I placed a brown Anolis in the box with its head projecting outward; then, leaving the lid off, I put the dark-box and the lizard in another and larger dark-box, which I thereupon closed. The lizard underwent the usual change, finally becoming green. On exposing the open box and lizard to daylight, the animal changed to brown. Thus the retention of the animal in the small dark-box by the cloth collar had no observable influence on the color changes.

After this test of my apparatus I proceeded to determine whether the light which fell on one part of the animal's body could be said to have any influence on the color changes in other non-illuminated portions. Six green animals were placed in the box in turn, each with its head outside and its trunk inside. All turned brown, on the trunk as well as on the head, though in every case a trace of green, for some unknown reason, remained on the neck, and in one instance one leg remained somewhat green. Notwithstanding these slight irregularities, this experiment showed beyond a doubt that the illumination of the head not only induces a change to brown in that region, but also in the part of the animal in the dark.

Next, six green lizards were placed in the box in turn, this time with the head inside and the trunk outside. Three animals changed wholly to brown excepting for some small spots on the neck, one changed partly to brown, and two remained green. Here, again, in three instances at least, the illumination of one part led to the appropriate color change in the non-illuminated part.

It might be concluded at once from these observations that the illuminated parts do influence the non-illuminated ones, and that therefore the change to brown is under the control of nerves. But it must be remembered that even with the best management of the apparatus some light always entered the dark-box through the open space between the animal's neck and the cloth collar, and that this light might be responsible for the change in the so-called non-illuminated part. To test this I put a green animal wholly in the dark-box, while another green one was placed in the opening with its head inside the box. Notwithstanding the fact that both head and trunk of the animal in the opening changed to brown, the introduced lizard remained green. When, under

similar circumstances, a brown lizard was introduced into the dark-box, it turned green. Moreover, when a brown animal was put inside the dark-box and the whole opening was left free for the entrance of light, the animal turned green and remained so, even in this diffuse light. There seems, therefore, no escape from the conclusion that the illumination of one part of the animal not only turns that part brown, but induces similar changes in the non-illuminated parts. I know of no way of explaining the induced changes except on the assumption that nerves serve as intermediate organs.

Why two of the animals in the original experiments remained green when their trunks were exposed to light, I do not know. It is possible that they were individuals whose reactions went on very slowly, and that I did not give them sufficient time; for when I tried these experiments I had not learned how slowly these changes sometimes come on. Or, since these two cases occurred among animals whose heads were in the dark, it is possible that they indicate that the condition of the head more easily impresses itself on that of the rest of the body than the reverse. I am, however, at present not in a position to decide this question.

The results of the experiments with the dark-box lead to two conclusions; first, that the nerve terminals in the skin of Anolis are sensitive to light and, secondly, that the change from brown to green may be brought about indirectly through nerves. That the nerve terminals in the skin of a vertebrate like Anolis should be sensitive to light is novel, so far as I know, and somewhat remarkable; but since a green animal with its head in the dark but its trunk in the light may change to brown all over, the evidence of this seems to me conclusive. It is, however, not without precedent, for in a recent paper Parker (:03) has shown, partly through the observations of others and partly through his own, that this property is possessed by several amphibians. From the evidence I have presented, I believe that the nerves of the skin in Anolis are sensitive to light.

If the change from green to brown is under the control of nerves, one should expect that the cutting of these nerves ought to prevent this change. Unfortunately the nerves are so small in Anolis that even those of the legs cannot be operated on with success, and hence a direct test of this proposition could not well be made. It is, however, possible to cut and destroy parts of the spinal cord. After such an operation one might expect to find the part of the body supplied by nerves from the destroyed portion of the cord incapable of changing to brown. To

test this I etherized some six or seven animals and destroyed the spinal cord by pithing from the middle trunk region posteriorly. After twenty-four hours, when the shock effects of the operation had probably passed off, I experimented on these animals by exposing them while in the green state to the light. Much to my surprise, in all cases the whole body, both in the regions with and those without cord, eventually turned brown; the portion with cord turned as in a normal animal, that without cord turned with less uniformity, though in the end it was indistinguishable from the part containing cord.

These results seemed at first sight to contradict the conclusion that the change from green to brown is controlled by nerves, but in reality they showed merely that the *spinal* nerves are not directly concerned with this change. Since it seems impossible to explain the conditions without assuming some nerves to be involved, and since the only other nerves present are the sympathetic, these observations point to the sympathetic fibres as the ones controlling the change from green to brown. When it is remembered that in mammals the muscles of the integumentary blood-vessels, and of the hair and the sweat glands are controlled by the sympathetic, this conclusion, that a change in the tegumentary chromatophores of Anolis is under the control of the same nerves, seems natural enough.

It is greatly to be regretted that Anolis carolinensis is so small that operations on the sympathetic ganglia, etc., such as have been carried out so extensively on mammals, could not have been resorted to in order to test this conclusion in detail; but such operations, though tried, were in the end abandoned. It is, however, well known, chiefly through the researches of Langley, that nicotine is a powerful poison, especially for the sympathetic ganglia. I therefore hoped to get localized effect by opening the body cavity of Anolis and painting certain ganglia with a solution of this drug; but here again, because of the small size of the lizard, my efforts were without avail. However, I experimented with subcutaneous injections and observed the influence of this drug on the color changes.

As a rule I injected under the skin of the flank of the lizard about $\frac{1}{65}$ of its body weight of nicotine solution. The strengths used were 1 per cent, $\frac{1}{10}$ per cent, and $\frac{1}{100}$ per cent. The first of these caused the animal to change from brown to green, induced a pronounced muscular trembling, and resulted very shortly in death. To show that these effects were not brought about by the simple operation of injection, a like amount of pure water was injected under the skin of several lizards

with the result that no color changes, muscular trembling, or other such disturbances were observed. The injection of $\frac{1}{10}$ per cent nicotine caused a change from brown to green and induced a slight muscular trembling, but the animals recovered in two or three hours in all respects except that the injection blister remained dark. With $\frac{1}{100}$ per cent nicotine no muscular trembling was observed, but the brown animals within one minute became green, and remained so for about three hours. When green animals were injected with either $\frac{1}{10}$ per cent or $\frac{1}{100}$ per cent nicotine, they remained green in the light about the same length of time as those did which were originally brown. It was remarkable that the side of the animal on which the injection was made remained green somewhat longer than the other side, and that all these animals changed to green, and remained so, in full daylight.

These results favor the conclusion already reached, that the change from green to brown is under the influence of the sympathetic nerves. Yet it might be maintained, so far as any facts thus far presented are concerned, that the action of the nicotine was not on the sympathetic system, but directly on the melanophores. That this is not so, may be shown in the following way. If the blood supply be cut off from any portion of the skin of Anolis, that part becomes green even when exposed to daylight, and will not turn brown from light stimulation until the blood is again freely admitted to the part. It follows from this, that since cutting off a piece of skin stops the flow of blood in it, one would expect such a piece to turn green, and such in fact is true. A piece of skin which by removal from the animal has become green, may, however, be made to assume the brown state by gently tapping it with a blunt instrument, provided it is kept on moist filter paper to prevent drying. The mechanical stimulation thus applied is sufficient to induce the temporary assumption of the brown condition, and demonstrates that the melanophores are not dead, but that they have simply drawn back their pigment. If now such a piece of skin is placed on filter paper moistened with nicotine solution and allowed to rest there till the solution has thoroughly permeated it, it will still, on being tapped, change to brown. Thus it is not the melanophores that have been changed, but the deeper mechanism through which the outward movement of their pigment is induced, and this, as we have already seen, is the sympathetic nervous system.

Although I was unsuccessful in my attempt to poison the sympathetic ganglia by local application, some evidence of local poisoning was obtained from the injection experiments. As I have already mentioned,

the side of an animal on which the injection is made always remains green longer than the opposite side. Since the nicotine does not act directly upon the melanophores, I believe this condition can be explained only on the assumption of a stronger poisoning effect on the sympathetic of the operated side than on the other. This is the only piece of evidence I have on the local action of the drug, but so far as it goes it indicates that, as in mammals, local action of the sympathetic would be followed by local reponse in the skin.

In none of my experiments have I seen the least evidence that light has any direct influence on the melanophores on entering or passing out of the brown state. If a piece of brown skin is cut off from an animal and quickly divided in two, one piece being put in the dark, and the other exposed to daylight, both turn green at the same rate, and apparently irrespective of the light. Many experiments of this kind have been tried, but without the least evidence of the direct action of light on the melanophores.

The change to the brown state is thus not influenced by the direct action of light. It may be brought about in at least two ways: first, through the direct stimulation of the melanophores by such mechanical means as tapping; and, secondly, through the indirect action of light, which stimulates the nerve terminals in the skin, and thereby induces through the sympathetic fibres an outward migration of the melanophore pigment.

3. *Green Condition.* — The change from brown to green, as has already been stated, invariably takes place when an animal is put into the dark, and requires on an average twenty-five minutes for its completion. Since the dark is due to the absence of the stimulus, light, and is not a stimulus itself, it would seem probable that the condition brought on in the dark corresponds to the unstimulated or resting state of the cell.

Much evidence has been obtained favoring this view, but before this is presented, a word must be said about two ways by which the green state may be brought about. As already noted, any means of stopping the circulation will invariably induce the green state in the portion of the skin affected. Thus, when a ligature is tied around the leg of an animal, so as to stop the flow of the blood, the leg soon becomes green. Since the animal reacts when the leg is touched, and moves the leg almost normally in locomotion, it cannot be said that the ligature has temporarily incapacitated the nerves. When the ligature is removed, the leg quickly becomes brown. The green condition, then, may be produced by cutting off the blood supply.

Secondly, when a brown Anolis is put in the dark, the skin becomes green. The transfer to the dark is certainly not accompanied with any cessation of the circulation, and I therefore believe that the change in this instance is dependent upon the withdrawal of the stimulus transmitted over the sympathetic nerves, which, as I have already shown, can call forth the brown state.

It follows from these observations that when a piece of skin is cut from an Anolis, the cause of the change to green may be either the loss of circulation, or the loss of nerve stimulus, or both, and that in experimenting these two factors must be kept constantly in mind. It is greatly to be regretted that these factors cannot readily be separated in experiments. Because of the small size of the animals it has been found impracticable to cut nerves without interfering with blood-vessels, or to ligate blood-vessels and leave the nerves uninjured, hence some important and interesting lines of experimental work have of necessity been abandoned.

In one direction, however, a definite conclusion has been reached. As already described, a ligated leg with the nerves functional, and the whole animal in the light, turns and remains green. This observation shows that, notwithstanding the influence of the nerves, the loss of circulation, even under conditions favorable for brown, is followed by the green state. The circulation, in other words, is a more important factor than the influence of nerves, and it is my belief that the change of a piece of excised skin to green is more dependent on the loss of circulation than on the loss of nerve connections.

It is interesting to observe that a piece of excised skin assumes the green color more quickly than the animal from which it comes. The following experiment illustrates this point. A normal Anolis was placed in the dark several times and found to change from brown to green in about twenty minutes. It was then kept in the light, and its right hind leg ligated so as to stop the circulation, but not to interfere with the use of this leg in locomotion and hence to leave its efferent nerve supply uninterrupted. Under these circumstances the leg changed from brown to green in about six minutes. The ligature was then removed and the animal was allowed to assume the brown condition, whereupon it was killed by decapitation and the leg previously ligated was immediately cut off. This leg became green in between three and four minutes. These observations show that the change to green comes on more quickly when both nerve action and circulation are interrupted, than when only the circulation is interrupted, and that, therefore, the sympathetic nerves

must be regarded as inhibiting slightly the change to the green state. The facts that ordinarily the brown condition is retained only in the light and that the change to the green state is somewhat inhibited when the nerves are intact, suggest that the sympathetic centres exert a tonus influence over the melanophore cells as long as the animal is exposed to daylight, and that the tonus only gradually subsides when the animal is placed in the dark.

The green state is not only produced by darkness, the withdrawal of the circulation, and possibly the cutting of nerves, but also in other ways. Most animals in narcosis from ether are green, and nicotine, as already mentioned, calls forth the green state. All animals change to green when they die. Thus the green state is in all cases the result of influences which either greatly reduce the stimuli or absolutely prevent them from reaching the melanophores. I therefore believe that the green state represents the resting condition of the melanophores — the state to which the cell returns on ceasing to receive stimuli. The brown state may be produced by direct mechanical stimulation of the melanophore cell, but I believe that usually it is the result of a continuous stimulation received from the sympathetic centres, which in turn are indirectly stimulated by light falling on some part of the animal's skin.

If this last conclusion is true, it follows that a brown animal, when placed with its head or its trunk in the dark-box, ought to show no change in color, for in each case enough of the body is exposed to light to keep up the sympathetic tonus for the whole integument. As a matter of fact such is the case; thus when each of six animals in the brown state were placed in the box, first with the head in, then with the trunk in, all remained brown.

From these various observations I conclude that the green state in Anolis represents the non-stimulated or resting state of its melanophores, and that this state is brought on by any means that wholly interrupts or greatly reduces the stimuli which naturally come to the melanophores. The change to this state is somewhat retarded by the partially inhibitory action of the sympathetic centres.

4. *Comparison with other Lizards.* — Enough has already been published on the color changes in lizards to show that these changes are not carried out in any uniform way in this group of animals. Thus in Chameleon, according to Brücke ('52), Keller ('95), and in fact all who in the last fifty years have examined the skin of this reptile carefully, the melanophores show an outward migration of pigment in the light and an inward migration in the dark. The same is true of Anolis. But in

Veranus, Uromastix, and Agame, according to Thilenius ('97), just the reverse takes place, — an inward migration in the light, and an outward one in the dark. That Thilenius's observations are probably correct is shown by the fact that similar conditions have been described by Filippi ('66) for Stellio, and by Wiedersheim (Hoffmann, '90), for Phrynosoma. Apparently lizards fall into two classes in this respect: those with an outward migration of pigment in the light, and those with an inward migration under like circumstances.

Although Anolis agrees with Chameleon in that its melanophore pigment moves outward in the light and inward in the dark, it differs fundamentally in other respects. I believe I have found satisfactory evidence to show that the outward migration of pigment in Anolis is dependent on the action of the sympathetic centres. The investigations of Brücke ('52), Bert ('75), Keller ('95), and others on the effects of nerve cutting and local stimulation by light in Chameleon have shown conclusively that the outward migration in this form is independent of nerves and due to the direct stimulation of the melanophore cells by light. On the other hand the inward migration of pigment in Anolis is a return of the cell to a resting state, dependent neither upon direct stimulation nor nerves, while in Chameleon this change has been shown beyond a doubt (Keller, '95, p. 137) to depend upon nerves. Thus, so far as the relation of the color changes to nerves is concerned, Chameleon and Anolis are the reverse of each other.

From the experiments of Brücke ('52), Bert ('75), Krukenberg ('80), and Keller ('95) on cutting the spinal cord of Chameleon, it would seem, since the skin became black posterior to the cut, that the spinal nerves control the inward migration of the pigment in this reptile. As already shown, such an operation in Anolis has little or no effect on the final change of color, and hence the sympathetic nerves must be assumed to act in Anolis.

Thus in a second fundamental particular Anolis differs from Chameleon. Anolis also differs not only from Chameleon, but also from all other lizards, so far as is known, in that the migration of the pigment in its melanophores is not directly influenced by light. It has been abundantly shown that the outward migration of the melanophore pigment in Chameleon is dependent upon the direct action of light, and Thilenius ('97, p. 539) has maintained that direct light stimulation also occurs in Varanus, Uromastix, and Agame. Nothing of this kind has been observed in Anolis. Here the inward migration might be suspected to occur under local stimulation, but it takes place in the dark

as well as in the light, and, as already shown, gives not the least evidence of direct stimulation of the melanophores.

Thus in three fundamental respects the pigment changes in Anolis differ from those in Chameleon, and suggest separate lines of phylogenetic differentiation, even though all such changes are to be traced back to the responses to light of the simpler form of tegumentary chromatophores.

IV. Summary.

1. The skin of Anolis carolinensis may be made to assume one or other of two extreme colors, dark-brown and pea-green.

2. The brown state for animals in confinement is taken on in daylight, and is produced by the outward migration of pigment granules from the bodies of the melanophores into their processes and ultimate branches.

3. The outward migration is accomplished in about four minutes.

4. It may be induced either by the mechanical stimulation of the skin or by the action of the sympathetic nerve centres.

5. The brown state is ordinarily maintained through a tonus established by the sympathetic nerves and dependent upon the stimulation of the nervous end-organs in the skin of Anolis by light.

6. The melanophores of Anolis are not stimulated directly by light.

7. The green state is taken on in the dark.

8. It is produced by the inward migration of the pigment granules of the melanophores, whereby the reflecting ochrophore layer becomes exposed to light.

9. The inward migration is accomplished in about twenty-five minutes.

10. It may be induced by any means which brings the melanophores into an unstimulated state: darkness, cessation of circulation, ether narcosis, nicotine poisoning, and possibly the cutting of nerves.

11. The green state represents the state of rest for the melanophore cells.

Bibliography.

Bert, P.

'75. Sur le mécanisme et les causes des changements de couleur chez le Caméléon. C. R. Acad. Sci., Paris, tom. 81, pp. 938–941.

Brücke, E.

'52. Untersuchungen über den Farbenwechsel des afrikanischen Chamäleons. Denkschr. kais. Akad. Wiss., Wien. Math.-Naturw. Cl., Bd. 4, Abt. 1, pp. 179–210, 1 Taf.

Cope, E. D.
:**00**. The Crocodilians, Lizards, and Snakes of North America. Ann. Rep. Smithsonian Inst. 1898; U. S. Nat. Mus. pp. 153–1270, pls. 1–36.

Filippi, F. de.
'66. Sulla struttura della cute dello Stellio Caucasicus. Mem. Accad. Sci., Torino, ser. 2, tom. 23, pp. 363–373, 1 tav.

Hoffmann, C. K.
'90. Eidechsen und Wasserechsen. Bronn's Klassen und Ordnungen des Thier-Reichs, Bd. 6, Abt. 3, Reptilia 2, pp. 443–1399, Taf. 49–107.

Keller, R.
'95. Ueber den Farbenwechsel des Chamaeleons und einiger anderer Reptilien. Arch. ges. Physiol, Bd. 61, pp. 123–168, Taf. 4.

Krukenberg, C. F. W.
'80. Ueber die Mechanik des Farbenwechsels bei Chamaeleon vulgaris, Cuv. Vergleichend-physiologische Studien, Reihe 1, Abt. 3, pp. 23–65.

Lockwood, S.
'76. The Florida Chameleon. Amer. Nat., vol. 10, No. 1, pp. 4–16.

Parker, G. H.
:**03**. The Skin and the Eyes as Receptive Organs in the Reactions of Frogs to Light. Amer. Jour. Physiol., vol. 10, No. 1, pp. 28–36.

Pouchet, G.
'76. Des changements de coloration sous l'influence des nerfs. Jour. Anat. et Physiol. norm. et path., tom. 12, pp 1–90, 113–165, pls. 1–4.

Thilenius, G.
'97. Der Farbenwechsel von Varanus griseus, Uromastix acanthinurus und Agame inermis. Morph. Arb., Bd. 7, Heft. 3, pp. 515–544, Taf. 17–18.

Wiedersheim, R.
(See Hoffmann, C. K.)

EXPLANATION OF PLATE.

The three figures are reproductions from photomicrographs taken by Mr. W. C. Greene from sections of the skin of *Anolis carolinensis* Cuv. All figures are magnified 200 diameters.

Abbreviations.

drm.	Derma.	*och'ph.*	Ochrophore.
e'drm.	Epidermis.	*st.crn.*	Horny layer.
mela'ph.	Melanophore.	*st.muc.*	Mucous layer.

Fig. 1. Section of a scute, perpendicular to its surface, from a piece of skin in the brown state. The branched processes of the melanophores are shown filled with pigment out to the epidermis.

Fig. 2. Tangential section of a scute from a piece of skin in the brown state.

Fig. 3. Section of a scute, perpendicular to its surface, from a piece of skin in the green state. The melanophore processes from which the pigment has migrated proximally may be traced, even in the photograph, almost to the epidermis.

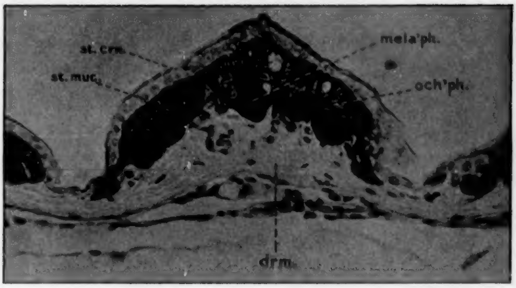

Fig. 1.

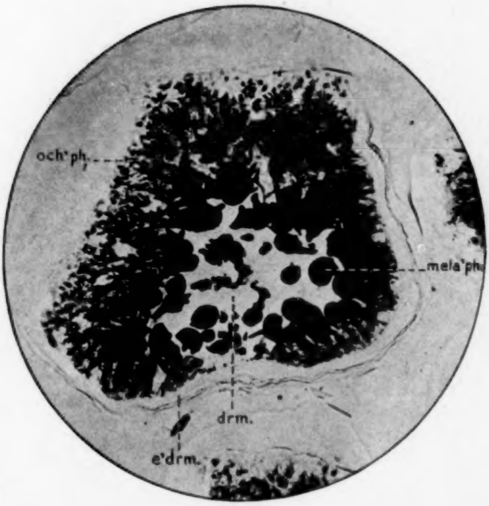

Fig 2.

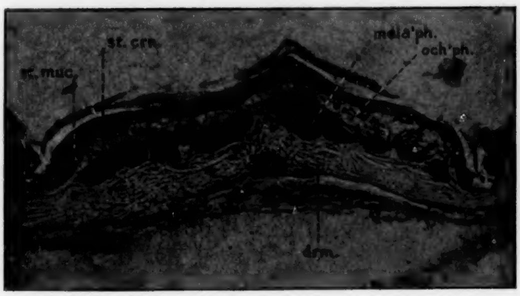

Fig. 3.